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Review

In vivo distribution of single chain variable fragment (scFv) against atherothrombotic oxidized LDL/β_2-glycoprotein I complexes into atherosclerotic plaques of WHHL rabbits: Implication for clinical PET imaging

Takanori Sasaki [a], Kazuko Kobayashi [a], Shoichi Kita [b], Kazuo Kojima [b], Hiroyuki Hirano [c], Lianhua Shen [a], Fumiaki Takenaka [a], Hiromi Kumon [a,d], Eiji Matsuura [a,e,*]

[a] Collaborative Research Center for OMIC, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
[b] IVD Development Department, Medical and Biological Laboratories Co, Ltd., Ina, Japan
[c] SHI Accelerator Service Ltd., Tokyo, Japan
[d] Department of Urology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan
[e] Department of Cell Chemistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan

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ABSTRACT

Background: Oxidized LDL (oxLDL) can exist as a complex with β_2-glycoprotein I (β_2GPI) in plasma/serum of patients with non-autoimmune atherosclerotic disease or antiphospholipid syndrome (APS). Nonetheless, direct *in vivo* evidence supporting the pathophysiological involvement of oxLDL/β_2GPI complexes and specific autoantibody against the complexes in developing atherothrombosis has yet been established. In the present study, we demonstrated *in vivo* distribution of single chain variable fragment of IgG anti-oxLDL/β_2GPI complexes (3H3-scFv) in Watanabe heritable hyperlipidemic (WHHL) rabbits by PET/CT imaging.
Methods: An antibody-based PET probe, ^{64}Cu-3H3-scFv, was established, and WHHL rabbits were applied for a non-autoimmune atherosclerotic model to demonstrate *in vivo* distribution of the probe.
Results: 3H3-scFv has exhibits specificity towards β_2GPI complexed with oxLDL but neither a free form of β_2GPI nor oxLDL alone. Post-intravenous administration of ^{64}Cu-3H3-scFv into WHHL rabbits has demonstrated a non-invasive approach for *in vivo* visualization of atherosclerotic lesion. The imaging probe achieved ideal blood clearance and distribution for optimal imaging capacity in 24 h, significantly shorter than that of an intact IgG-based imaging probe. ^{64}Cu-3H3-scFv targeted on atherosclerotic plaques in aortas of WHHL rabbits where extensive accumulation of lipid deposits was observed by lipid staining and autoradiography. The accumulation of ^{64}Cu-3H3-scFv in aortic segments of WHHL rabbits was 2.8-folds higher than that of controls ($p = 0.0045$).
Conclusions: The present *in vivo* evidence supports the pathophysiological involvement of oxLDL/β_2GPI complexes in atherosclerotic complications of WHHL rabbits. ^{64}Cu-3H3-scFv represents a novel PET imaging probe for non-invasive pathophysiological assessment of oxLDL/β_2GPI complexes accumulated in atherosclerotic plaques.

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* Corresponding author at: Collaborative Research Center for OMIC and Department of Cell Chemistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan.
E-mail address: eijimatu@md.okayama-u.ac.jp (E. Matsuura).

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1. Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of heterogeneous antiphospholipid antibodies that closely associate with clinical manifestations such as arterial and venous thromboembolic events [1–3]. β_2-glycoprotein I (β_2GPI) is among the most relevant antigenic targets of pro-thrombotic antiphospholipid antibodies characteristically found in patients with the APS [4–8]. β_2GPI can bind to oxLDL to form a highly immunogenic oxLDL/β_2GPI complexes [9–11]. Both oxLDL/β_2GPI complexes and their immune complexes formed with APS-derived autoantibodies have been implicated in the development of so-called "autoimmune-mediated" atherothrombosis. We have previously developed an IgG monoclonal antibody (mAb) specific to oxLDL/β_2GPI complexes, namely, "WB-CAL-1", derived from non-immunized APS mice model, NZW × BXSB F1 male mice (W/B F1 mice) [12]. In addition, oxLDL/β_2GPI complexes have also been demonstrated in non-autoimmune human atherosclerotic plaques by immunohistochemical staining [13]. As such we hypothesized that intravenous injection of WB-CAL-1antibody or antibody similar specificity will bind to oxLDL/β_2GPI complexes and accumulates atherosclerosis plaques. Coupled with *in vivo* imaging techniques, such application can be used as a comprehensive and non-invasive diagnosis platform to evaluate the severity of atherosclerotic lesion by assessing the distribution and amount of oxLDL/β_2GPI complexes.

Diagnostic imaging of atherosclerotic lesions represents an important tool [14] and computed tomography (CT) is commonly used to determine the presence of ectopic calcified plaques [15,16] and invasive angiographic procedures are required to evaluate the degree of arterial stenosis [17]. Herein, special effort has been aimed to develop *in vivo* systems capable of detecting vulnerable plaques associated with adverse cardiovascular events [18,19]. To date, various pro-atherogenic molecules are being tested as potential target for molecular imaging technologies in identification of vulnerable plaques [20,21]. Several groups have looked into adhesion molecules, oxLDL and angiogenic/atherogenic factors as potential molecular imaging biomarkers to detect atherosclerotic lesions [22–26]. Early event of atherosclerosis has been associated with the accumulation of LDL in the sub-endothelial matrix [27], and oxidative modification of these accumulated LDL subsequently triggered systemic and localized inflammations. Therefore, it is now commonly accepted that oxLDL plays a central role in the progression of atherosclerosis [28].

During the past decade, more sophisticated and non-invasive techniques to detect non-stenotic vascular lesions and plaques susceptible to rupture are necessary to implement early treatment to prevent further disease progression. However, there is still controversy regarding the use of even non-invasive imaging in high risk subjects who do not exhibit any cardiovascular symptom such as chest pain or electrocardiogram abnormalities [29]. PET with different kinds of positron emitters were applied for imaging atherosclerotic plaques [30,31]. Especially radiolabeling antibody against to several molecules in atherosclerosis had been developed. In the present study, we generated a mouse mAb, namely 3H3, that recognizes which human oxLDL/β_2GPI complexes and not β_2GPI alone. We further prepared the 25 kDa scFv by constructing the fragment with the identical sequences of complementarity determining regions (CDR) and framework regions of 3H3 IgG. The 3H3-scFv was labeled with ^{64}Cu via 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) for PET imaging and the generated probe was then introduced intravenously into the atherosclerosis animal model, Watanabe heritable hyperlipidemic (WHHL) rabbits to evaluate its *in vivo* accumulation in atherosclerotic lesions.

2. Materials and methods

2.1. Establishment of a hybridoma secreting IgG anti-oxLDL/β_2GPI complexes

BALB/c female mice (6 week-old) were immunized with human oxLDL/β_2GPI complexes [32] (50 μg/head) and emulsified with Freund's complete adjuvant repeatedly. Three days after the final injection, lymph-node cells were collected and fused with myeloma cells (P3-X63Ag8U1). Screenings for hybridomas that secretes antigen-specific antibody were carried out by ELISA using human oxLDL/β_2GPI complexes- or β_2GPI-coated 96-well microtiter plates (see Section 2.4 for details). A full length of IgG mAb was established.

2.2. Cloning of the IgG-cDNA

mRNAs were obtained from the hybridoma cells by using illustra™ QuickPrep Micro mRNA Purification Kit (GE Healthcare, Little Chalfont, UK). The mRNAs were then converted into cDNA using the First-Strand cDNA Synthesis kit (GE Healthcare) and further amplified by PCR.

2.3. Design of single-chain variable fragment (scFv)

The 3H3-scFv was designed by inserting the glycine (G)-serine (S)-rich 15-amino-acid linker (GGGGSGGGGSGGGGS) in between variable light-chain (VL) and variable heavy-chain (VH) regions. The 3H3-scFv with six-histidine tag (IDT, San Jose, CA)-gene was then ligated into an expression vector, pcDNA3.1 (Life technologies, Foster City, CA). The Chinese hamster ovary (CHO-K1) cells were transfected with linearized pcDNA3.1 vector by electroporation and a stable cell line was established. The cell line was cultured in the serum free-medium, CD-CHO (Life technologies). 3H3-scFv was purified from culture supernatant